



## A HISTOLOGICAL AND CYTOPHOTOMETRIC STUDY OF THE EFFECTS OF X-RAYS ON THE MOUSE TESTIS<sup>1</sup>

JOHN H. D. BRYAN AND JOHN W. GOWEN

*Department of Genetics, Iowa State College, Ames, Iowa*

Studies of Bergonié and Tribondeau (1904), Regaud and Blanc (1906) and others focussed attention on irradiation-induced changes in reproductive organs and gametes of various organisms. Their findings indicate that spermatogonia are the most x-ray-sensitive components of the seminiferous epithelium and that, following the decrease in numbers of spermatogonia, the other cell types disappear in the order of their development. The topic has been the further subject of numerous publications and the literature has been reviewed by Heller (1948).

Investigations of the nature of x-ray-induced sterility in the male mouse have been carried out by Snell (1933) and by Hertwig (1938). Recent reviews by Kaufmann (1954) and Russell (1954) have dealt with radiation-induced changes of cytological and genetic interest.

Recently, emphasis has been given to quantitative histological studies of irradiated testes. Studies have been carried out by Eschenbrenner, Miller and Lorenz (1948), Eschenbrenner and Miller (1950), Fogg and Cowing (1951), Barrow and Tullis (1952), Shaver (1953) and Oakberg (1955). Investigations have been facilitated by the histological scoring procedure developed by Chalkley (1943) and by the cytological studies of Roosen-Runge and Giesel (1950), and of Leblond and Clermont (1952).

The present approach differs from the above in that it represents the combination of a quantitative histological method together with cytophotometry, thus affording data with respect to the DNA content of irradiated tissues as well as changes in the frequency of cells.

### MATERIALS AND METHODS

Two inbred strains of mice, differing in their sensitivity to mouse typhoid, were used in these experiments. The animals chosen were 58-day old males of strains BALB/Gw (hereinafter designated as Ba) and S. The animals were irradiated in plastic tubes and were exposed to a dose of 320 r (250 pkv, 30 ma; filtration 0.25 mm. Cu, 1 mm. Al; anode-target distance 47.5 cm., dose rate 430 r/min.). The irradiation was delivered to the pelvic region only, the rest of the body being shielded with lead.

Control and irradiated animals were killed at 1, 8 and 24 hours, 3, 5, 10, 16 and 28 days after irradiation. From each animal one testis was removed, quickly freed of associated tissues, rolled on filter paper, weighed on a Roller Smith torsion bal-

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ance and dropped into fixative. The other testis was removed and treated in a similar manner, and was later used for dry weight determinations. The testis in fixative was then cut into several pieces. Very few minutes elapsed from the time each animal was killed until the testis removed into fixative was subdivided.

In the case of the one-hour controls, kidney tissue also was fixed and later served as a control tissue for the Feulgen reaction.

### *Fixation*

All tissues were fixed in Carnoy's acetic-alcohol (1:3) for three hours, washed in several changes of absolute alcohol, cleared in benzene and embedded in 56–58° C. Tissuemat. Sections were cut at 7 and at 10  $\mu$ . For photometric purposes, sections from all animals of one strain were mounted on the same slide together with sections of kidney obtained from a Ba control animal.

### *Staining*

The desoxyribose nucleic acid (DNA) content of nuclei was visualized by means of the Feulgen reaction. The reagent was prepared according to the method of Stowell (1945) and sections were stained for two hours at room temperature following hydrolysis (12 minutes) in 1 N HCl at 60° C. Some Feulgen preparations were lightly counterstained with fast green and used for cytological studies. Unhydrolyzed slides were immersed in the Feulgen reagent and served as controls for photometric purposes.

Other slides were stained with a mixture of safranin and fast green as described by Bryan (1955). These preparations were used for routine cytological and histological studies as well as for the quantitative scoring procedure described below.

Additional slides were stained by means of the periodic acid-Schiff (PAS) reaction. The reagents were prepared according to McManus (1948) and the slides were stained as described by Bryan (1954), methyl green being used as a counterstain. This procedure was used, in the earlier phases of this work, as an aid in the identification of the developmental stages of the seminiferous epithelium, after the manner described by Leblond and Clermont (1952). It should be mentioned, however, that the stages of development of the seminiferous epithelium as described for the rat do not entirely correspond to the stages observed in the mouse.

### *Photometry*

Measurements of the DNA-Feulgen complex were made with an apparatus essentially the same as that described by Pollister (1952) and others. A tungsten filament lamp (6 volt, 18 amp.) was used as a light source and the spectral lines isolated by means of a Perkin-Elmer monochromator equipped with appropriate accessory optics.

It was necessary to compare data obtained from several slides. Therefore, sections of control kidney were used as a standard to check on the reproducibility of the Feulgen reagent. Means of the DNA-Feulgen content of kidney nuclei obtained from the different slides did not differ significantly from each other.

Transmittance data were obtained by the "plug" method described by Swift

TABLE I<sup>2</sup>  
*Frequency of cell types at various times after 320 r of x-rays*

Stage and strain		1 hour	8 hours	1 day	3 days	5 days	10 days	16 days	28 days
Spermatogonia in interphase	Ba	102.6	110.3	91.0	1.1	9.2	10.4	50.1	161.3
	S	107.8	101.5	79.2	2.2	0.0	3.3	37.7	187.6
Spermatogonia in mitosis	Ba	82.9	26.8	65.9	4.9	12.2	9.8	56.1	173.2
	S	102.6	11.5	21.5	0.0	0.0	2.3	26.4	222.8
Spermatocyte chromatin	Ba	96.3	98.3	109.3	116.7	92.4	64.8	10.0	110.5
	S	96.3	101.6	107.6	112.1	97.2	82.8	0.0	122.2
Spermatid nuclei	Ba	95.1	97.0	87.3	93.4	122.5	144.4	126.4	49.8
	S	111.8	95.5	99.7	106.0	132.5	144.1	149.5	23.1
Sperm heads	Ba	112.8	105.2	113.8	134.7	146.6	228.4	369.7	51.6
	S	89.6	107.6	97.0	112.2	132.4	246.7	358.3	63.4
Sertoli nuclei	Ba	104.9	110.0	97.0	72.8	96.5	125.4	139.1	115.1
	S	99.3	85.9	104.7	113.4	125.5	160.4	125.9	136.6

<sup>2</sup> Values expressed as per cent of controls.

(1950), and were converted into extinction values ( $\log_{10} 1/T$ ). From the extinction data, the amounts of the DNA-Feulgen complex were computed as described by Swift (1950). In most cases the nuclei selected for measurement purposes were located beforehand and their location indicated on a camera lucida map.

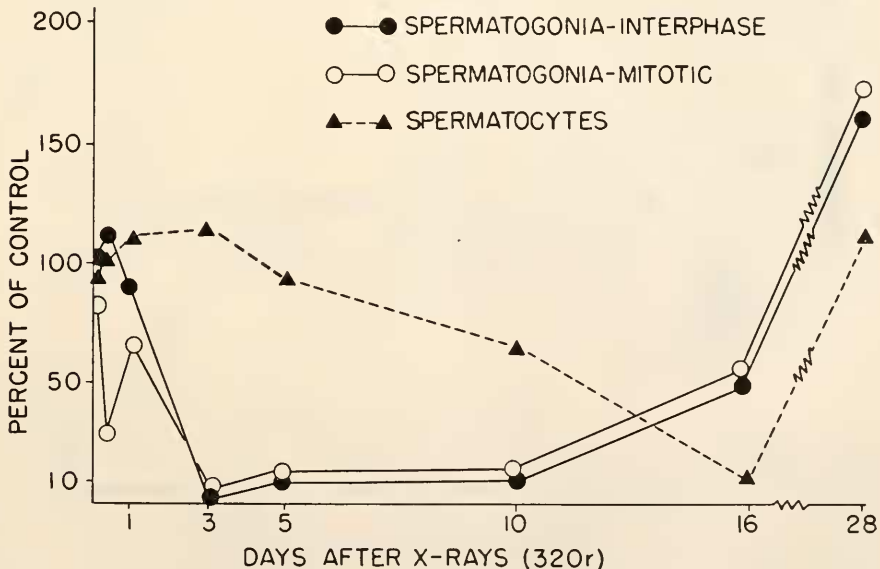


FIGURE 1. Strain Ba. Incidence of spermatogonia and spermatocytes at different times following 320 r of x-rays.

*Scoring of cells*

Estimates of the relative areas of the seminiferous tubules occupied by each stage of spermatogenesis were obtained by means of the quantitative scoring procedure developed by Chalkley (1943). The manner in which this procedure was employed was based on a similar application by Eschenbrenner, Miller and Lorenz (1948) but with minor modifications.

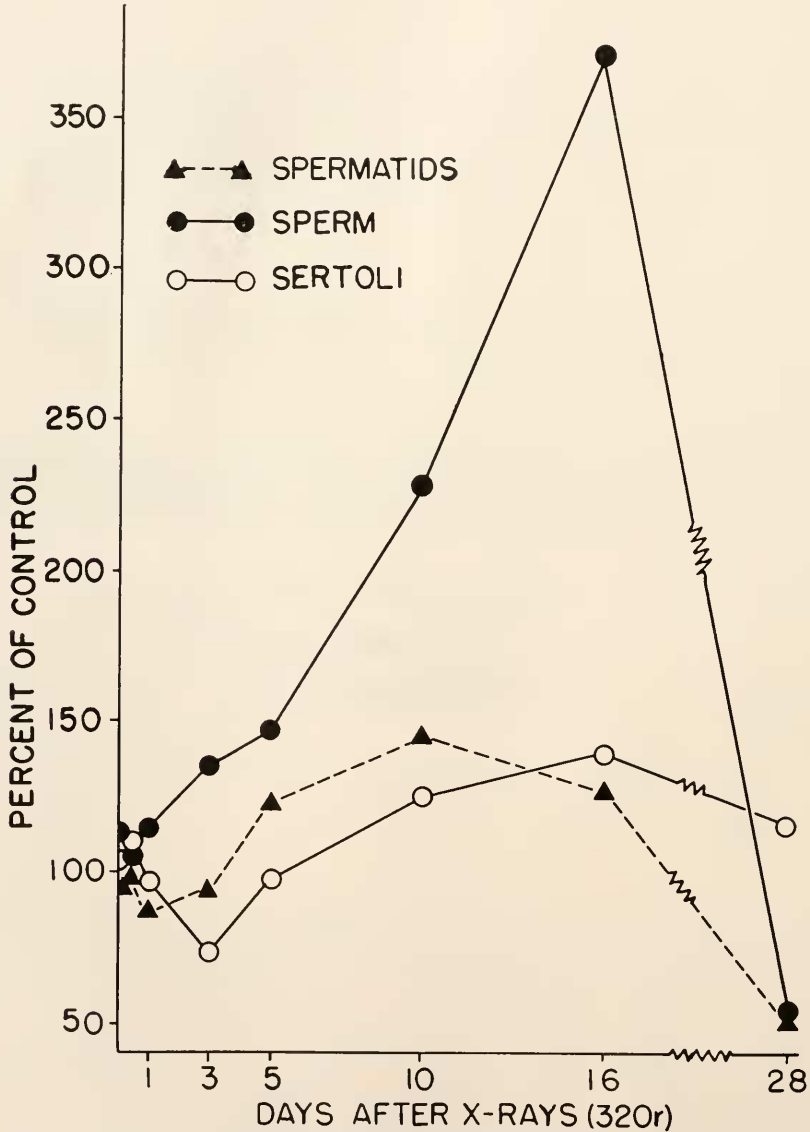


FIGURE 2. Strain Ba. Incidence of spermatids, sperm and Sertoli nuclei at different times following 320 r of x-rays.

Nuclei were recorded as belonging to the following classes: interphasic spermatogonia, dividing spermatogonia, spermatocytes, spermatids, sperm and Sertoli nuclei. Eschenbrenner *et al.* did not record "hits" on Sertoli nuclei and recorded only spermatids possessing spherical nuclei and only those sperm heads evincing "well defined dorsal fins." In the present work, all stages of spermatid development and of sperm maturations were recorded. On account of these differences in procedure, the results of Eschenbrenner *et al.* and those reported here are not strictly comparable.

## RESULTS

*A. Histological studies*

The histological scoring data are summarized in Table I and are presented in graph form in Figures 1-4. In Table I the data pertaining to both strains are

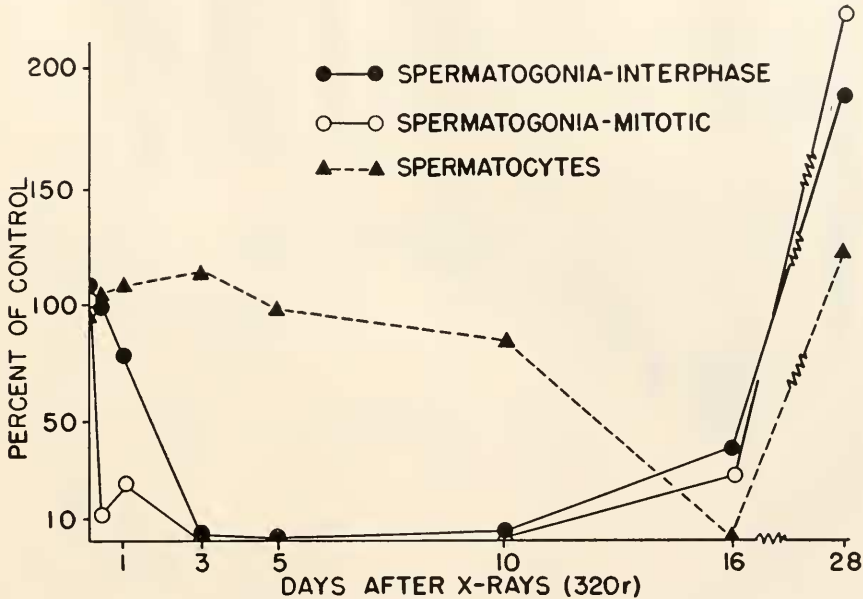


FIGURE 3. Strain S. Incidence of spermatogonia and spermatocytes at different times following 320 r of x-rays.

presented together in order to facilitate their comparison. All values in this table are expressed in terms of per cent of control values.

These data indicate that with the exception of the dividing spermatogonia, there is no pronounced change in the cellular composition of the seminiferous epithelium during the first 24 hours following irradiation. During the period of 1-3 days after irradiation there occurs a marked decline in the area occupied by interphasic spermatogonia. Some regeneration of spermatogonia is apparent by 10 days and is well underway by 16 days. Dividing spermatogonia, on the other hand, undergo a rapid decrease during the period of 1-8 hours after irradiation. At 24 hours,

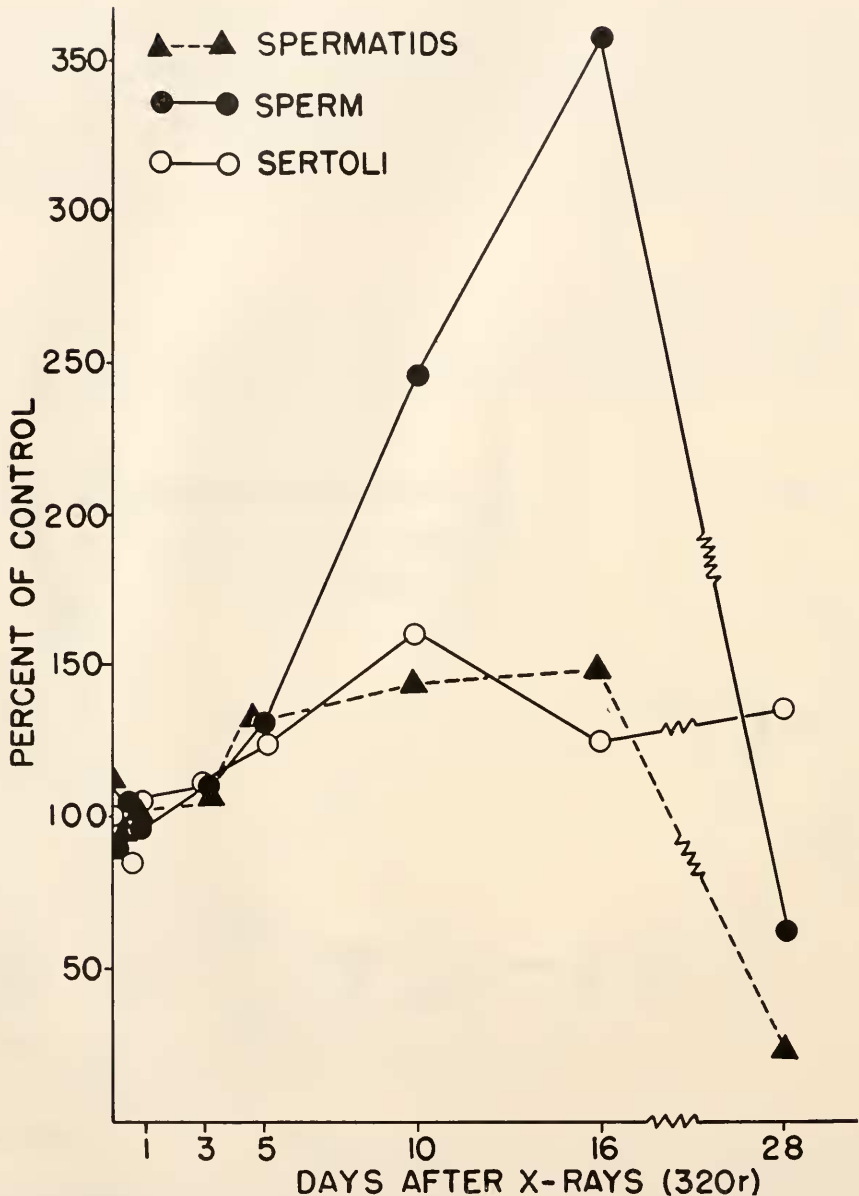


FIGURE 4. Strain S. Incidence of spermatids, sperm and Sertoli nuclei at different times following 320 r of x-rays.

the area occupied by these cells shows a moderate increase (from 26.8% of the control value at 8 hours to 65.9% of the control value at 24 hours in strain Ba, and from 11.5% to 21.5% in the case of strain S). This increase is only a transient feature since by three days the area occupied by dividing spermatogonia has further

declined to a level of less than 5% of the control value. Thereafter, the dividing spermatogonia show essentially the same behavior as the interphasic cells.

During the course of the histological studies it was found that the seminiferous epithelium of both control and irradiated animals contained cells in various stages of degeneration. The frequency of such cells appeared to be greater in the irradiated material taken from the animals killed during the early part of the post-irradiation period. Accordingly, the pertinent sections were analyzed by means of the Chalkley procedure and the data (expressed as percentages) obtained, from both strains, are presented in Table II.

### B. DNA-Feulgen

The data obtained are summarized in Tables III and IV. The values represent the mean amounts of the DNA-Feulgen complex (in arbitrary units) together with

TABLE II  
*Spermatogonial and non-spermatogonial necrosis after 320 r of x-rays*

Strain	Time after irradiation	% spermatogonial necrosis	% non-spermatogonial necrosis	% total necrosis <sup>3</sup>
Ba S	Control	3.75	2.07	2.27
		4.03	2.52	2.76
Ba S	1 hour	3.40	2.99	3.05
		5.76	2.37	2.83
Ba S	8 hours	9.71	2.24	2.98
		8.80	1.68	2.56
Ba S	24 hours	7.41	2.26	2.79
		4.55	2.21	2.36

$$^3 \frac{\text{No. of necrotic cells}}{\text{total cells scored}} \times 100.$$

their respective standard errors. Nuclei possessing the diploid amount of DNA are designated as Class II nuclei, those with twice this amount Class III, and Class IIa represents nuclei possessing an amount of DNA intermediate between these levels.

The range of values for the DNA classes was determined as follows. In the case of the control kidney tissue, the highest value obtained was found to be about 50% higher than the lowest (values ranged from 1.78 units to 2.69 units). The Feulgen-stained kidney nuclei have a more uniform appearance than the spermatogonial nuclei and consequently the spread of values in the latter is somewhat larger with amounts ranging from 1.91 units to 2.94 units. As an approximation to the range expected for Class III nuclei these values may be doubled, yielding a range of from 3.82 to 5.88 units. In actual fact the values for these nuclei were

found to range from 3.9 units to 5.6 units but with most of the nuclei distributed over a range of 4-5 units. Thus the spread of values is rather lower than expected. It may be accounted for on the basis of distributional error since many of these nuclei (which are slightly larger than Class II nuclei) show clumping of the stained material. Those values falling between the upper limit of Class II and the lower limit of Class III were assigned to Class IIa.

### C. Other observations

The irradiated material was examined for indications of damage to the chromosomes. No indications of extensive damage, at the cytological level, were seen.

TABLE III  
*DNA-Feulgen content of spermatogonia, strain Ba*

Tissue and treatment	Class	No. of nuclei	DNA-Feulgen content
Kidney—control	II	54	2.15±0.03
Testis—control	II	96	2.42±0.04
	IIa	71	3.51±0.04
	III	46	4.52±0.06
Testis—320 r 1 day	II	24	2.57±0.05
	IIa	24	3.44±0.05
3 days	(No nuclei suitable for measurement)		
5 days	II	31	2.52±0.05
	IIa	11	3.30±0.09
	III	1	4.32
10 days	II	29	2.52±0.05
	IIa	12	3.36±0.06
	III	7	4.26
16 days	II	52	2.28±0.07
	IIa	24	3.41±0.05
	III	4	4.16
28 days	II	38	2.54±0.05
	IIa	35	3.50±0.05
	III	7	4.30

The frequency of anaphase figures showing one or more bridges appeared to be very low. During the early period of regenerative activity "nests" of spermatogonial cells were occasionally seen containing one or (rarely) more anaphase bridges. The underlying mechanism responsible for this is not understood; it would appear that in some instances the irradiated spermatogonia must have divided at least once before the chromosomal aberrations showed up.

Other instances of cellular (or nuclear) damage were also observed. Thus, giant nuclei were found in some tubules of the irradiated material. These nuclei



made their appearance at about 5 days after irradiation and persisted until 16 days, reaching an apparent high point at 10 days. In addition a few of the tubules examined were found to contain cysts composed of 4 to 8 spermatogonial-like nuclei. The fate of these structures is unknown.

At 16 days after irradiation, spermatozoa occupy about 40% of the area of the seminiferous epithelium. It is of interest that some sperm heads present an abnormal appearance. In contrast to the controls, where the Feulgen-positive material (DNA) is distributed in a relatively homogeneous manner, the abnormal sperm

TABLE IV  
*DNA-Feulgen content of spermatogonia, strain S*

Tissue and treatment	Class	No. of nuclei	DNA-Feulgen content
Kidney (Ba)—control	II	30	2.11±0.04
Testis—control	II	40	2.46±0.05
	IIa	47	3.41±0.04
	III	13	4.29±0.07
Testis—320 r 1 day	II	37	2.66±0.04
	IIa	46	3.34±0.03
	III	2	4.73
3 days	(No nuclei suitable for measurement)		
5 days	(No nuclei suitable for measurement)		
10 days	II	21	2.36±0.09
	IIa	6	3.57
16 days	II	44	2.48±0.05
	IIa	24	3.37±0.04
	III	2	4.22
28 days	II	53	2.50±0.05
	IIa	25	3.26±0.03
	III	2	4.20

heads have a "bubbly" structure. Such sperm may have been derived from irradiation-damaged spermatids or, possibly, spermatocytes.

#### DISCUSSION

In addition to histological and genetic approaches, the effects of irradiation on biological systems have been studied from the biochemical viewpoint with the aid of tracer techniques. Thus Holmes (1947) used  $P^{32}$  in his studies of the effect of x-rays on the synthesis of nucleic acids in the rat Jensen sarcoma. He found that irradiation markedly inhibited the uptake of  $P^{32}$  into the DNA fraction of the tumor. Studies by Hevesy (1949) have suggested that the delay in mitosis following irradiation is related to the marked inhibition of DNA synthesis, as

measured by  $C^{14}$  uptake into DNA purines. It has been shown also, by Abrams (1951), using glycine-1- $C^{14}$ , that irradiation greatly reduces the incorporation of radioactive carbon into the DNA and PNA purines of rat and rabbit intestine, whereas the synthesis of proteins is relatively unaffected. Similarly, Smellie *et al.* (1955) also find marked inhibition of  $P^{32}$  incorporation into the nucleic acids of normally mitotically active rabbit tissues following irradiation with 1000 r of x-rays.

Recent work by Klein and Forsberg (1954), using the Ehrlich mouse ascites tumor, indicates that 1250 r of x-rays completely suppresses mitotic activity for a period of 16 hours without any increase in the cell death rate. A linear increase of cell volume with time followed the irradiation treatment. By chemical means, these workers showed that PNA and total nitrogen per cell increased at nearly the same rate as cell volume; DNA, however, did not show this behavior. Later studies by Forsberg and Klein (1954), using glycine-2- $C^{14}$ , point in the same direction, that is, that irradiation brings about a marked decline in DNA synthesis, and, simultaneously, inhibits mitotic activity.

With the aid of a cytophotometer it is possible to measure the DNA-Feulgen content of individual spermatogonial nuclei in interphase. The data presented in Tables III and IV show that in the controls three classes of nuclei are present—those possessing the diploid amount of DNA-Feulgen complex (Class II), the tetraploid amount (Class III) and nuclei containing an intermediate amount of DNA (Class IIa). This latter group is representative of those nuclei engaged in the reduplication of their chromatin content in preparation for the next division, whereas Class III represents spermatogonial nuclei in which synthesis is complete, while the changes involved in the initiation of prophase have not yet occurred.

Irradiation brings about an inhibition of DNA synthesis, as measured by the uptake of radioactive precursor molecules. Hence, after irradiation, it would be expected that those nuclei which had been prevented from reduplicating their DNA content would be unable to enter mitosis. The net result should be loss, through mitosis, of the Class III nuclei. During the recovery process, DNA synthesis recommences and Class III nuclei should be produced. The DNA-Feulgen data presented in Tables III and IV are in agreement with these ideas. Thus, in the control measurements, Class III nuclei are relatively abundant, whereas shortly after irradiation, they are either absent or very much reduced in number.

It is not possible to determine directly whether or not the first spermatogonia to enter mitosis, after irradiation, are those which, though possessing the requisite amount of chromosomal material, had been prevented from dividing. However, the cytophotometric data strongly suggest that this is the case. The reason is that if such nuclei were prevented from entering mitosis then it should be expected that at 24 hours after irradiation a significant number should be present. The data presented in Tables III and IV show that no Class III nuclei were present in the sample obtained from strain Ba and only two such nuclei (2.4% of the sample) were encountered in the case of strain S.

It might be expected that at 28 days after irradiation, when the frequency of spermatogonial nuclei is much higher than in the controls, that a large proportion of these nuclei should prove to belong to Class III. However, the dry weights of irradiated testes at this time comprise only 34–47% of the control weights. These facts, together with the photometric data, may be taken to suggest that the high

rate of mitosis is of a compensating nature and that the spermatogonia enter mitosis immediately after the reduplication of the chromatin is completed.

As shown by Bullough (1948), mitotic activity in the mouse testis remains about constant and at a high level throughout the day. According to the previously mentioned tracer studies, irradiation should bring about a marked decline in the mitotic rate of the seminiferous epithelium (and hence in the area of the tubules occupied by mitotic figures). Reference to Table I and Figures 1 and 3 indicates that at one hour after irradiation, the area of the tubules occupied by dividing spermatogonia is not very different from the control value in the case of strain S, and somewhat lower in the case of strain Ba. By 8 hours, the level has fallen to about 27% of the control in the case of strain Ba, and to 12% in strain S. However, at the present time one cannot rule out the possibility that the mitotic activity has undergone a more rapid decline prior to 8 hours, by which time some regeneration is underway. That this is the case might, perhaps, receive some support from the findings that the one-day values show a substantial increase over the 8-hour levels (from 26.8% of the control value at 8 hours to 65.9% of the control value at 24 hours, or an increase of 140%, in the case of strain Ba; from 11.5% to 21.5%, or an increase of 87%, in the case of strain S).

The duration of mitotic delay (temporary inhibition) as a function of dose has been investigated by Canti and Spear (1929), Henshaw (1932, 1940) and others. (See Lea, 1955, for a discussion of this point.) While the period of delay, for a given dose, varies with the different organisms used, the findings allow the generalization that, for 300 r, recovery would be expected to commence at about 6–8 hours after irradiation. The results reported here for the mouse testis are in fair agreement with this estimate.

The stage at which cells are most sensitive to irradiation has been investigated by Spear (1935), Carlson (1940, 1941, 1954), Creighton (1941) and others. Creighton (1941) has reported the most sensitive stage for grasshopper spermatogonia to be three hours prior to anaphase, that is, at late interphase or early in prophase. The results of Carlson (1941), working with grasshopper neuroblasts, indicate the critical stage to be in prophase, while Spear (1935) concludes from his tissue-culture experiments that late interphase is the most sensitive stage. The work of Henshaw (1940) and others on *Arbacia* eggs suggests prophase to be the most sensitive stage.

The differences noted above are probably not fundamental, but merely represent differences due to the conditions of the experiments in question. The main point is that the most sensitive stage appears to be that part of the mitotic cycle lasting from late interphase to early prophase. Irradiation at or immediately prior to this stage causes the cells to be held up, the duration of this mitotic delay being dependent upon the dose received.

At the time of irradiation, the further away a cell is from this critical stage in its development, the less it is retarded. Similarly, cells which have progressed beyond this stage, at the time of irradiation, do not undergo inhibition of mitosis. These observations afford a partial explanation for the present findings—that the rise in the mitotic rate during the period of 8–24 hours after x-rays is followed by a further and more extensive decline.

In the testis, blockage of spermatogonial mitoses would be expected to result

in maturation depletion of the tubules since the spermatogonial cells would be unable to undergo the necessary divisions to renew the proportion of cell types lost (through maturation). The present results confirm this view.

The suppression of DNA synthesis produced by irradiation appears to be induced in cells which were irradiated in interphase since cytophotometric investigations by various workers (see for instance Swift, 1950 and Bryan, 1951) indicate that reduplication of the DNA content, in preparation for the next division, takes place during this phase of the mitotic cycle. Further support for this conclusion may be drawn from the work of Gentcheff and Gustafsson (1939). The findings of these authors suggest that irradiation-induced inhibition of mitosis is caused by the prevention or delay of chromosomal reduplication and consequently the sensitivity of cells to x-rays is correlated with the time of chromosome reproduction.

It is evident that irradiation of cells and tissues brings about specific effects at different levels of organization. Thus at the molecular level there are, for example, such effects as the denaturation of SH-group-containing proteins, and disturbances of the systems responsible for the synthesis of the nucleic acids. At the cytological level, diverse chromosomal observations are encountered as well as effects resulting in the prolongation of prophase (or late interphase). Then there appear to be various latent cellular effects which become evident at a later date—in some instances one or more cell generations later.

It should be expected that a number of cells would receive lethal doses of radiation resulting in immediate death or death prior to the completion of a division cycle. It then follows that if sufficient spermatogonia are destroyed in this manner, maturation depletion of the tubules would ensue. The data of Oakberg (1955) strongly support this hypothesis. However in the present work, as can be seen from Table II, the proportion of necrotic spermatogonia constitutes only a minor fraction of the total. This work therefore supports the alternative hypothesis; that is, irradiation-induced maturation depletion of the seminiferous epithelium is brought about mainly through the irradiation-induced changes responsible for the inhibition of mitotic activity, with cell death playing only a minor role in this process. These conclusions, based on histological and cytophotometric data, therefore do not support the findings of Oakberg (1955), but instead, offer further confirmation of the findings of Eschenbrenner and Miller (1950).

In conclusion, then, the data presented in this paper, with respect to irradiation-induced maturation depletion of the seminiferous tubules, do not support the spermatogonial cell death hypothesis. Instead the data are consistent with the view that the major effects of irradiation are (a) the suppression of mitosis due to inhibition of DNA synthesis and (b) the suppression of mitosis due to the prolongation of prophase through the disturbance of other physiological and biochemical processes necessary for normal mitotic activity.

#### SUMMARY

1. Changes in the seminiferous epithelium, induced by 320 r of x-rays, have been analyzed by means of a quantitative histological procedure.
2. With the aid of a cytophotometer, measurements have been made of the DNA-Feulgen content of control and irradiated spermatogonial nuclei.

3. The histological data indicate that irradiation, with 320 r of x-rays, results in a temporary maturation depletion of the seminiferous epithelium. This depletion is brought about through the inhibition of spermatogonial mitosis rather than irradiation-induced spermatogonial necrosis. The frequency of spermatogonia reaches its lowest point by three days after irradiation and regeneration does not begin until after 10 days.

4. The cytophotometric data suggest that irradiation-induced inhibition of DNA synthesis is a contributing factor with respect to the suppression of mitotic activity.

## LITERATURE CITED

- ABRAMS, R., 1951. Effect of X-rays on nucleic acid and protein synthesis. *Arch. Biochem.*, **30**: 90-99.
- BARROW, J., AND J. L. TULLIS, 1952. Sequence of cellular responses to injury in mice exposed to 1000 r total-body X-radiation. *Arch. Pathol.*, **53**: 391-407.
- BERGONJÉ, J., AND L. TRIBONDEAU, 1904. Action des rayons X sur le testicule du rat blanc. *C. R. Soc. Biol.*, **57**: 592-595.
- BRYAN, J. H. D., 1951. DNA-protein relations during microsporogenesis of *Tradescantia*. *Chromosoma*, **4**: 369-392.
- BRYAN, J. H. D., 1954. Cytological and cytochemical studies of oogenesis of *Popilius disjunctus* Illiger (Coleoptera—Polyphaga). *Biol. Bull.*, **107**: 64-79.
- BRYAN, J. H. D., 1955. Differential staining with a mixture of safranin and fast-green FCF. *Stain Technol.*, **30**: 153-157.
- BULLOUGH, W. S., 1948. Mitotic activity in the male mouse, *Mus musculus* L. The diurnal cycles and their relation to waking and sleeping. *Proc. Roy. Soc. Lond., Ser. B*, **135**: 212-233.
- CANTI, J. G., AND F. G. SPEAR, 1929. The effect of gamma radiation on cell division in tissue culture *in vitro*. Part II. *Proc. Roy. Soc. Lond., Ser. B*, **105**: 93-98.
- CARLSON, J. G., 1940. Immediate effects of 250 r of X-rays on the different stages of mitosis in neuroblasts of the grasshopper, *Chortophaga viridifasciata*. *J. Morph.*, **66**: 11-23.
- CARLSON, J. G., 1941. Effects of X-radiation on grasshopper chromosomes. *Cold Spring Harbor Symp. Quant. Biol.*, **9**: 104-111.
- CARLSON, J. G., 1954. Immediate effects on division, morphology and viability of the cell. *In: Radiation biology*, edited by A. Hollaender, Vol. I, Part II, McGraw-Hill Book Company, New York.
- CHALKLEY, H. W., 1943. Method for the quantitative morphologic analysis of tissues. *J. Nat. Cancr Inst.*, **4**: 47-53.
- CREIGHTON, M., 1941. The effects of X-rays on mitotic and meiotic chromosomes and cell division in *Chorthippus longicornis* (Orthoptera). *J. Exp. Zool.*, **87**: 347-369.
- ESCHENBRENNER, A. B., E. MILLER AND E. LORENZ, 1948. Quantitative histologic analysis of the effect of chronic whole-body irradiation with gamma rays on the spermatogenic elements and the interstitial tissues of the testes of mice. *J. Nat. Cancr Inst.*, **9**: 133-148.
- ESCHENBRENNER, A. B., AND E. MILLER, 1950. Effect of roentgen rays on the testis: Quantitative histological analysis following whole-body exposure of mice. *Arch. Pathol.*, **50**: 736-749.
- FOGG, L. C., AND R. F. COWING, 1951. The changes in cell morphology and histochemistry of the testis following irradiation and their relation to other induced testicular changes. I. Quantitative random sampling of germinal cells at intervals following direct irradiation. *Cancr Res.*, **11**: 23-28.
- FORSSBERG, A., AND G. KLEIN, 1954. Studies on the effects of X-rays on the biochemistry and cellular composition of ascites tumors. II. Changes in the pattern of glycine- $2\text{-C}^{14}$  incorporation during the first two hours after irradiation *in vivo*. *Exp. Cell Res.*, **7**: 480-497.
- GENTCHEFF, G., AND A. GUSTAFSSON, 1939. The double chromosome reproduction in *Spinacia* and its causes. *Hereditas*, **25**: 371-386.

- HELLER, M., 1948. The testis. *In: Histopathology of irradiation from external and internal sources*, edited by W. Bloom. McGraw-Hill Book Company, New York.
- HENSHAW, P. S., 1932. Studies of the effect of roentgen rays on the time of first cleavage in some marine invertebrate eggs. I. Recovery from roentgen-ray effects in *Arbacia* eggs. *Amer. J. Roent. Rad. Therapy*, **27**: 890-898.
- HENSHAW, P. S., 1940. Further studies on the action of roentgen rays on the gametes of *Arbacia punctulata*. *Amer. J. Roent. Rad. Therapy*, **43**: 899-912.
- HERTWIG, P., 1938. Die Regeneration des Sameneithels der Maus nach Rontgenbestrahlung, unter besonderer Berücksichtigung der Spermatogonien. *Arch. exp. Zellforsch.*, **22**: 68-73.
- HEVESY, G., 1949. Effect of X-rays on the incorporation of C<sup>14</sup> into deoxyribonucleic acid. *Nature*, **163**: 869-870.
- HOLMES, B. E., 1947. The inhibition of ribo and thymonucleic acid synthesis in tumour tissue by irradiation with X-rays. *Brit. J. Radiol.*, **20**: 450-453.
- KAUFMANN, B. P., 1954. Chromosome aberrations induced in animal cells by ionizing radiation. *In: Radiation biology*, edited by A. Hollaender, Vol. I, Part II, McGraw-Hill Book Company, New York.
- KLEIN, G., AND A. FORSSBERG, 1954. Studies on the effect of X-rays on the biochemistry and cellular composition of ascites tumors. I. Effect on growth rate, cell volume, nucleic acid and nitrogen content in the Ehrlich ascites tumor. *Exp. Cell Res.*, **6**: 211-220.
- LEA, D. E., 1955. Actions of radiations on living cells. Second ed., edited by L. H. Gray. The University Press, Cambridge, England.
- LEBLOND, C. P., AND Y. CLERMONT, 1952. Spermiogenesis of rat, mouse, hamster and guinea pig as revealed by the "periodic acid-fuchsin sulfurous acid" technique. *Amer. J. Anat.*, **90**: 167-215.
- MCMANUS, J. F. A., 1948. Histological and histochemical uses of periodic acid. *Stain Technol.*, **23**: 99-108.
- OAKBERG, E. F., 1955. Sensitivity and time of degeneration of spermatogenic cells irradiated in various stages of maturation in the mouse. *Radiation Res.*, **2**: 369-391.
- POLLISTER, A. W., 1952. Photomultiplier apparatus for microspectrophotometry of cells. *Lab. Investigation*, **1**: 106-114.
- REGAUD, C. L., AND J. BLANC, 1906. Action des rayons X sur les diverses générations de la lignée spermatique. Extrême sensibilité des spermatogonies à ces rayons. *C. R. Soc. Biol.*, **61**: 163-165.
- ROOSEN-RUNGE, E. C., AND L. O. GIESEL, 1950. Quantitative studies on spermatogenesis in the albino rat. *Amer. J. Anat.*, **87**: 1-30.
- RUSSELL, W. L., 1954. Genetic effects of radiation in mammals. *In: Radiation biology*, edited by A. Hollaender, Vol. I, Part II, McGraw-Hill Book Company, New York.
- SHAVER, S. L., 1953. X-irradiation injury and repair in the germinal epithelium of male rats. I. Injury and repair in adult rats. *Amer. J. Anat.*, **92**: 391-432.
- SMELLIE, R. M. S., G. F. HUMPHREY, E. R. M. KAY AND J. N. DAVIDSON, 1955. The incorporation of radioactive phosphorus into the nucleic acids of different rabbit tissues. *Biochem. J.*, **60**: 177-193.
- SNELL, G. D., 1933. X-ray sterility in the male house mouse. *J. Exp. Zool.*, **65**: 421-441.
- SPEAR, F. G., 1935. Tissue culture and radiological research. *Brit. J. Radiol.*, **8**: 68, 280.
- STOWELL, R. E., 1945. Feulgen reaction for thymonucleic acid. *Stain Technol.*, **20**: 45-56.
- SWIFT, H. H., 1950. The desoxyribose nucleic acid content of animal nuclei. *Physiol. Zool.*, **23**: 169-198.